

SUPPLEMENTAL MATERIAL

The morphogenic protein CopD controls the spatio-temporal dynamics of PBP1a and PBP2b in *Streptococcus pneumoniae*

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Anaïs Pelletier and Cassandra Lenoir contributed equally to this work and are co-first authors.

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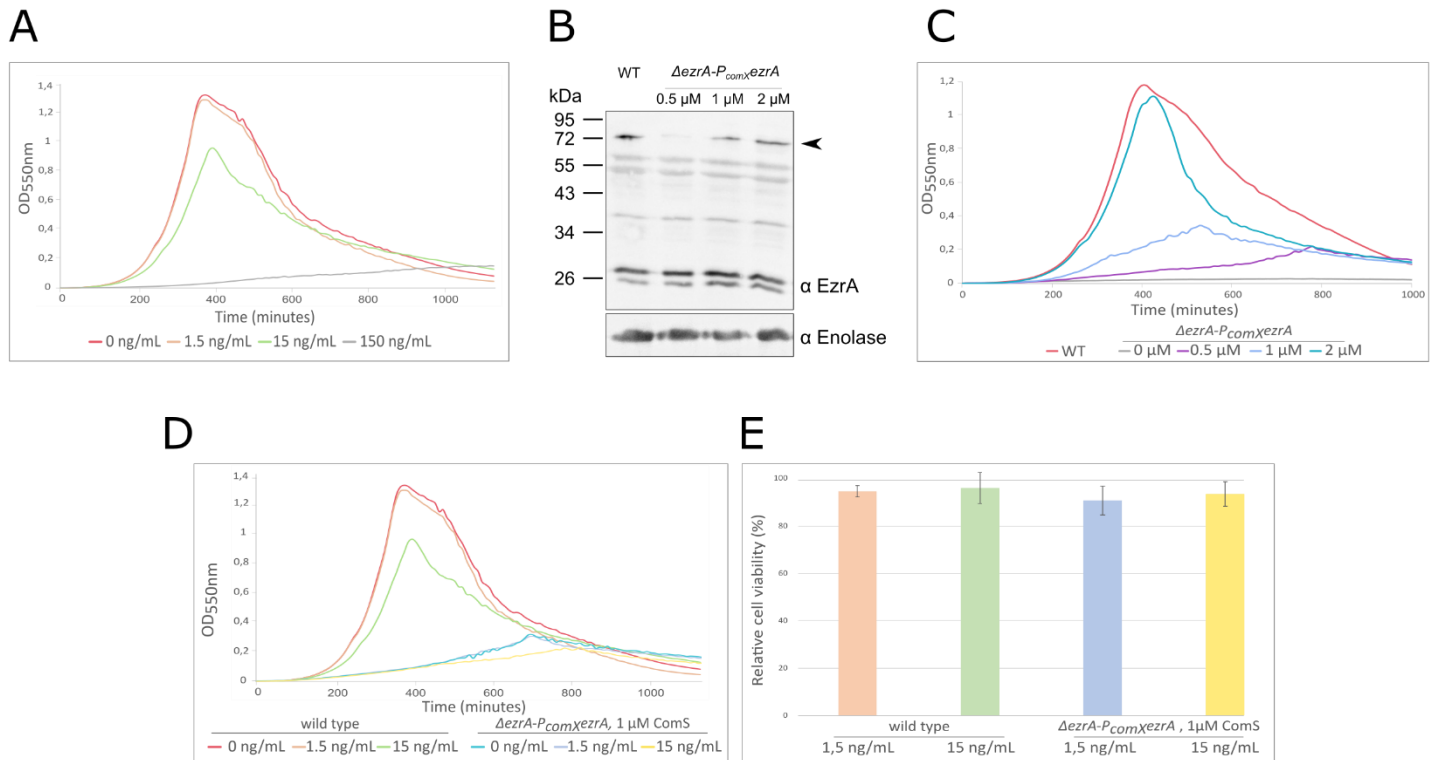


Figure S1: Effect of tetracycline and/or *ezrA*-depletion on cell growth and viability. (A)

WT cells were grown in C+Y medium at 37°C in the presence of increasing concentration of tetracycline. The OD₅₅₀ was read automatically every 10 min. **(B)** Western immunoblot of whole-cell lysates from $\Delta ezrA$ - P_{comX} -*ezrA* cells, grown to exponential phase in the presence (0.5, 1 or 2 μ M) of the ComS inducer, were probed with specific anti-EzrA (α EzrA) antibody. To estimate the relative quantity of proteins in crude extract and to compare the different lanes, we used enolase (Spr1036) as an internal standard. The enolase was detected using specific antibodies (α Enolase) and is presented in the lower part of the Figure. EzrA is indicated with a black arrow. **(C)** $\Delta ezrA$ - P_{comX} -*ezrA* cells were grown in C+Y medium at 37°C in the absence or presence of decreasing concentration of ComS from 2 to 0 μ M. The OD₅₅₀ was read automatically every 10 min. **(D)** Growth curves of WT and $\Delta ezrA$ - P_{comX} -*ezrA* strains (+ 1 μ M ComS) in presence of 1.5 ng/ml or 15 ng/ml of tetracycline. **(E)** Relative cell viability of $\Delta ezrA$ - P_{comX} -*ezrA* (+ 1 μ M ComS) compared to WT cells in the presence of 1.5 ng/ml or 15 ng/ml of tetracycline. Bar chart represents the mean \pm SEM.

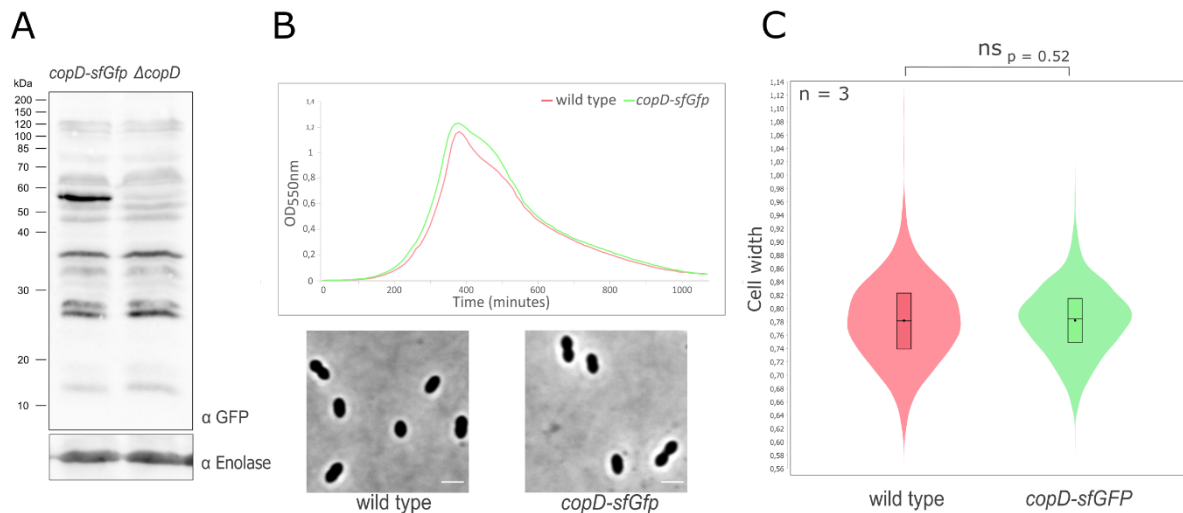


Figure S2: Expression and functionality of the CopD-sfGFP fusion. (A) The Western immunoblot was probed with specific anti-GFP antibodies (α GFP) to determine *copD* expression in *copD-sfGfp* cells. To estimate the relative quantity of proteins in crude extract and to compare the different lanes, we used enolase (Spr1036) as an internal standard. The enolase was detected using specific antibodies (α Enolase) and is presented in the lower part of the Figure. (B) Growth of WT and *copD-sfGfp* strains. Strains were grown in C+Y medium at 37°C in a spectrophotometer. The OD₅₅₀ was read automatically every 10 min. Representative phase contrast microscopy images of WT and *copD-sfGfp* cells are shown below the growth curves. Scale bar, 2 μ m. (C) Corresponding violin plot show the distribution of the cell width as determined using MicrobeJ (56). The distribution of the cell width is shown in red for the WT strain and in green for the *copD-sfGfp* strain. The box indicates the 25th to the 75th percentile, and the whiskers indicate the minimum and the maximum values. The mean and the median are indicated with a dot and a line in the box, respectively. Statistical comparison was done using t-test. n=3 indicates the number of independent experiments with a total of 8000 cells analyzed.

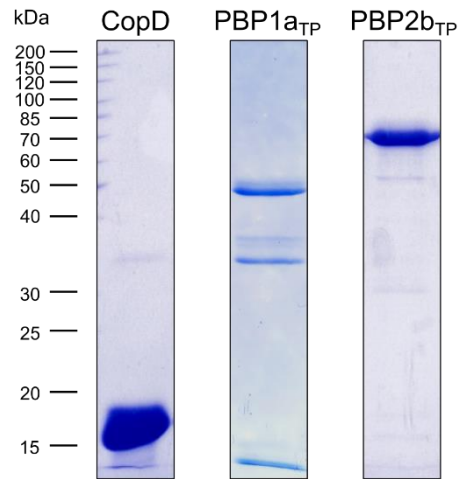


Figure S3: Purification of CopD_{ED}, PBP1A_{TP} and PBP2b_{TP}. Proteins were overproduced in *E. coli* BL21 as 6his-tagged fusion proteins. After purification using a Ni-NTA resin, purified proteins were analyzed by SDS-PAGE.

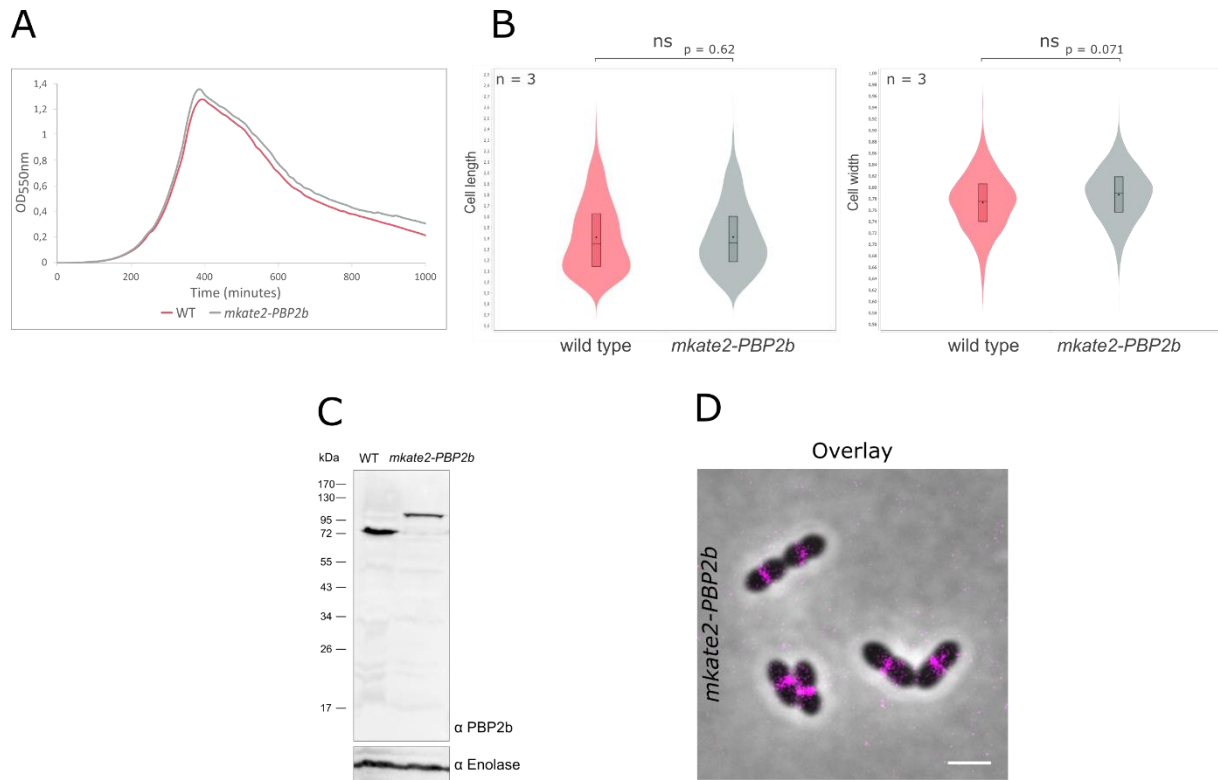


Figure S4: Validation of the *mkate2-PBP2b* fusion. (A) Growth curves of WT and *mkate2-PBP2b* cells in C+Y medium at 37°C. The OD₅₅₀ was read automatically every 10 min. (B) Violin plot showing the distribution of the cell length (left panel) and cell width (right panel) for WT (red) and *mkate2-PBP2b* (grey) strains as determined using MicrobeJ (56). The box indicates the 25th to the 75th percentile, and the whiskers indicate the minimum and the maximum values. The mean and the median are indicated with a dot and a line in the box, respectively. Statistical comparison was done using t-test. n=3 indicates the number of independent experiments with a total of 10 000 cells analyzed. (C) Expression of *mkate2-PBP2b*. *mkate2-PBP2b* represents the only source of PBP2b from its endogenous chromosomal locus. After SDS-PAGE and electro-blotting, the membrane was probed with anti-PBP2b antibody. As control, WT cells were also analyzed. (D) Overlays between phase-contrast and mKate2 images of *mkate2-PBP2b* cells. Scale bar, 2 μm.

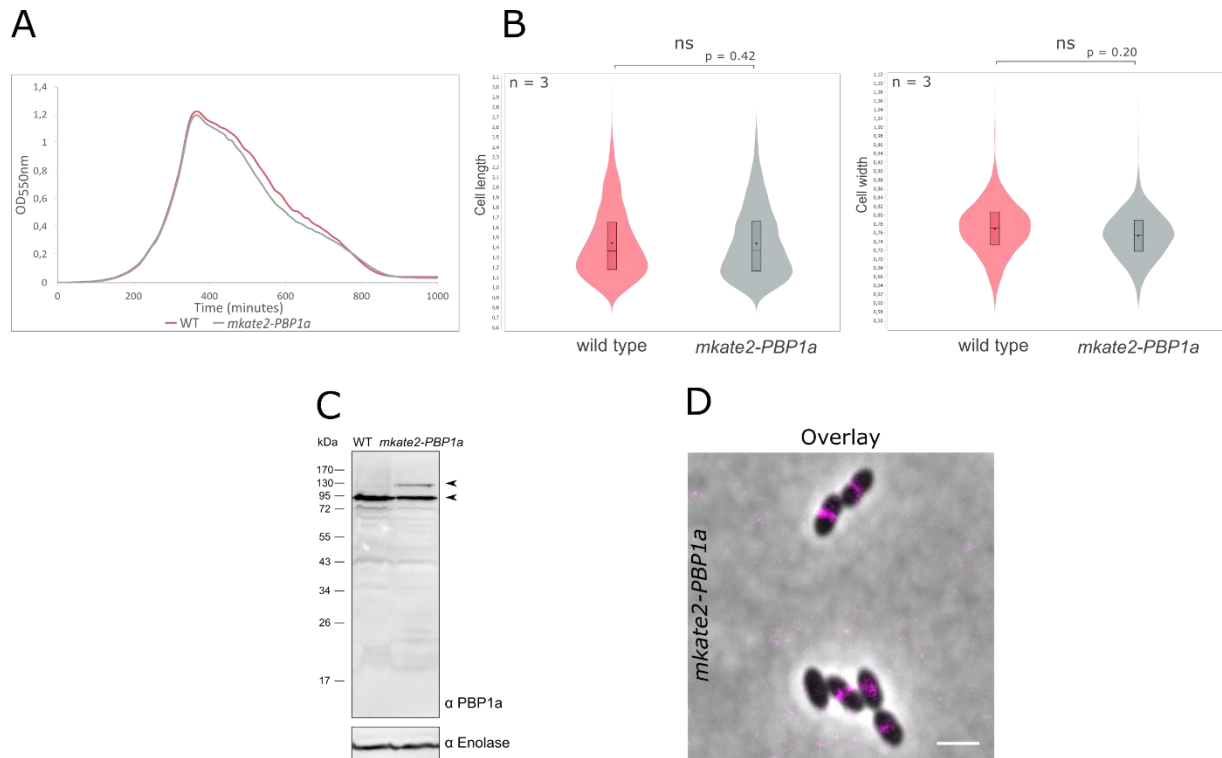


Figure S5: Validation of the *mkate2-PBP1a* fusion. (A) Growth curves of WT and P_{Zn} -*mkate2-PBP1a* cells in C+Y medium at 37°C. The OD₅₅₀ was read automatically every 10 min. (B) Violin plot showing the distribution of the cell length (left panel) and cell width (right panel) for WT (red) and *mkate2-PBP1a* (grey) strains as determined using MicrobeJ (56). The box indicates the 25th to the 75th percentile, and the whiskers indicate the minimum and the maximum values. The mean and the median are indicated with a dot and a line in the box, respectively. Statistical comparison was done using t-test. n=3 indicates the number of independent experiments with a total of 10 000 cells analyzed. (C) Expression of *mkate2-PBP1a*. The merodiploid strain P_{Zn} -*mkate2-PBP1a* expresses the chromosomal *PBP1a* and the ectopic *mkate2-PBP1a* fusion under the control of the zinc-inducible P_{Zn} promoter. The two forms are indicated by arrows. After SDS-PAGE and electro-blotting, the membrane was probed with anti-PBP1a antibody. As control, WT cells were also analyzed. (D) Overlays between phase-contrast and *mkate2* images of P_{Zn} -*mkate2-PBP1a* cells. Scale bar, 2 μ m.

Table S1 - Strains and plasmids used in this study

Strain	Genotype and description	Reference	Primers (Table S2)
<i>S. pneumoniae</i> strains			
R800	<i>S. pneumoniae</i> R6 derivative	Gift from J.-P. Claverys, Toulouse, France	
Wild type	R800 <i>rpsL1</i>	Gift from J.-P. Claverys, Toulouse, France	
<i>copD</i> :: <i>Janus</i>	R800 <i>rpsL1</i> , <i>copD</i> :: <i>kan-rpsL</i> ; <i>Kan</i> ^R	This study	1, 2, 3, 4, 5, 6
Δ <i>copD</i>	R800 <i>rpsL1</i> , Δ <i>copD</i> ; <i>Str</i> ^R	This study	1, 2, 7, 8
<i>copD</i> - Δ PepSY	R800 <i>rpsL1</i> , <i>copD</i> - Δ PepSY(A57-L162); <i>Str</i> ^R	This study	1, 2, 9, 10
<i>copD</i> -sfGfp	R800 <i>rpsL1</i> , <i>copD</i> -sfGfp; <i>Str</i> ^R	This study	1, 2, 10, 11, 12, 13
<i>P</i> _{comX} - <i>copD</i>	R800 <i>rpsL1</i> , Δ IS1167::P1::P _{comR} - <i>comR</i> , <i>cpsN-O</i> ::P _{comX} - <i>copD</i> ; <i>Str</i> ^R	This study	14, 15, 16, 17, 18, 19
<i>copD</i> :: <i>Janus</i> - <i>P</i> _{comX} - <i>copD</i>	R800 <i>rpsL1</i> , Δ IS1167::P1::P _{comR} - <i>comR</i> , <i>cpsN-O</i> ::P _{comX} - <i>copD</i> , <i>copD</i> :: <i>kan-rpsL</i> ; <i>Kan</i> ^R	This study	1, 2
Δ <i>copD</i> - <i>P</i> _{comX} - <i>copD</i> ,	R800 <i>rpsL1</i> , Δ IS1167::P1::P _{comR} - <i>comR</i> , <i>cpsN-O</i> ::P _{comX} - <i>copD</i> , Δ <i>copD</i> ; <i>Str</i> ^R	This study	1, 2
<i>P</i> _{comX} -sfGfp	R800 <i>rpsL1</i> , Δ IS1167::P1::P _{comR} - <i>comR</i> , <i>cpsN-O</i> ::P _{comX} -sfGfp; <i>Str</i> ^R	This study	14, 15, 16, 17, 20, 21
<i>gfp</i> -PBP2b	R800 <i>rpsL1</i> , <i>gfp</i> -PBP2b; <i>Str</i> ^R	This study	22, 23, 24, 25, 26
<i>copD</i> :: <i>Janus</i> - <i>gfp</i> -PBP2b	R800 <i>rpsL1</i> , <i>gfp</i> -PBP2b, <i>copD</i> :: <i>kan-rpsL</i> ; <i>Kan</i> ^R	This study	1, 2
Δ <i>copD</i> - <i>gfp</i> -PBP2b	R800 <i>rpsL1</i> , <i>gfp</i> -PBP2b, Δ <i>copD</i> ; <i>Str</i> ^R	This study	1, 2
<i>P</i> _{Zn} - <i>gfp</i> -PBP1a	R800 <i>rpsL1</i> , <i>bgaA</i> ::P _{Zn} - <i>gfp</i> -PBP1a; <i>Str</i> ^R <i>Tet</i> ^R	Gift from C. Morlot	
<i>copD</i> :: <i>Janus</i> - <i>P</i> _{Zn} - <i>gfp</i> -PBP1a	R800 <i>rpsL1</i> , <i>bgaA</i> ::P _{Zn} - <i>gfp</i> -PBP1a, <i>copD</i> :: <i>kan-rpsL</i> ; <i>Kan</i> ^R <i>Tet</i> ^R	This study	1, 2
Δ <i>copD</i> - <i>P</i> _{Zn} - <i>gfp</i> -PBP1a	R800 <i>rpsL1</i> , <i>bgaA</i> ::P _{Zn} - <i>gfp</i> -PBP1a, Δ <i>copD</i> ; <i>Str</i> ^R <i>Tet</i> ^R	This study	1, 2
<i>copD</i> :: <i>Janus</i> - <i>gfp</i> -PBP2b	R800 <i>rpsL1</i> , <i>gfp</i> -PBP2b, <i>copD</i> :: <i>kan-rpsL</i> ; <i>Kan</i> ^R	This study	1, 2
<i>gfp</i> -ftsA	R800 <i>rpsL1</i> , <i>gfp</i> -ftsA; <i>Str</i> ^R	1	
<i>copD</i> -sfGfp- <i>gfp</i> -PBP2b	R800 <i>rpsL1</i> , <i>gfp</i> -PBP2b, <i>copD</i> -sfGfp; <i>Str</i> ^R	This study	1, 2
<i>P</i> _{Zn} -mkate2-PBP1a	R800 <i>rpsL1</i> , <i>P</i> _{Zn} -mkate2-PBP1a, <i>Str</i> ^R <i>Tet</i> ^R	Gift from C. Morlot	
<i>copD</i> :: <i>Janus</i> - <i>P</i> _{Zn} -mkate2-PBP1a	R800 <i>rpsL1</i> , <i>P</i> _{Zn} -mkate2-PBP1a, <i>copD</i> :: <i>kan-rpsL</i> , <i>Kan</i> ^R <i>Tet</i> ^R	This study	1, 2
<i>copD</i> -sfgfp- <i>P</i> _{Zn} -mkate2-PBP1a	R800 <i>rpsL1</i> , <i>P</i> _{Zn} -mkate2-PBP1a, <i>copD</i> -sfgfp, <i>Str</i> ^R <i>Tet</i> ^R	This study	1, 2

<i>mkate2-PBP2b</i>	<i>R800 rpsL1, mkate2-PBP2b, Str^R</i>	This study	22, 23, 25, 27, 28
<i>copD::Janus-mkate2-PBP2b</i>	<i>R800 rpsL1, mkate2-PBP2b, copD::kan-rpsI, Kan^R</i>	This study	1, 2
<i>copD-sfGfp-mkate2-PBP2b</i>	<i>R800 rpsL1, mkate2-PBP2b, copD-sfGfp, Str^R</i>	This study	1, 2
<i>P_{comX}-ezrA</i>	<i>R800 rpsL1, ΔIS1167::P1::P_{comR}-comR, cpsN-O::P_{comX}-ezrA, Str^R</i>	This study	14, 15, 16, 17, 29, 30
<i>ezrA-Janus</i>	<i>R800 rpsL1, ezrA-kan-rpsI; Kan^R</i>	This study	3, 4, 31, 32, 33, 34
<i>ezrA-Janus-P_{comX}-ezrA</i>	<i>R800 rpsL1, ΔIS1167::P1::P_{comR}-comR, cpsN-O::P_{comX}-ezrA, ezrA-kan-rpsI; Kan^R</i>	This study	31, 32
<i>ΔezrA-P_{comX}-ezrA</i>	<i>R800 rpsL1, ΔIS1167::P1::P_{comR}-comR, cpsN-O::P_{comX}-ezrA, ΔezrA; Str^R</i>	This study	31, 32, 35, 36
<i>E. coli</i> strains			
<i>XL1-Blue</i>	<i>supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac-F'[proAB + lac^IlacZΔM15 Tn10 (Tc^R)]</i>	2	
<i>BTH101</i>	<i>F-, cya-99, araD139, galE15, galk16, rpsL1 (Str^R), hsdR2, mcrA1, mcrB1</i>	Euromedex	
<i>BL21 (DE3)</i>	<i>F-, ompT gal dcm lon hsdS_B(r_B⁻m_B⁻) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])</i>	3	
<i>BL21 star (DE3)</i>	<i>F⁻ ompT gal dcm lon hsdS_B(r_B⁻m_B⁻) λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB⁺]_{K-12}(λ^S)</i>	Invitrogen	
Plasmids			
<i>pETPhos-copD (extra)</i>	<i>pETPhos derivative, encoding CopD, from Met38 to Leu162, Amp^R</i>	This study	37, 38
<i>pETPhos-PBP2b (extra)</i>	<i>pETPhos derivative, encoding PBP2b, from Met39 to Asn685, Amp^R</i>	This study	39, 40
<i>pETPhos-PBP1a (extra)</i>	<i>pETPhos derivative, encoding PBP1a, from Ser37 to Pro719, Amp^R</i>	This study	41, 42
<i>pKT25</i>	<i>P_{lac}-T25- kan pACYC ori</i>	4	
<i>pUT18C</i>	<i>P_{lac}-T18-, bla</i>	4	
<i>pKT25-zip</i>	<i>P_{lac}-T25-leucine zipper region from yeast GCN4. kan pACYC ori</i>	4	
<i>pUT18C-zip</i>	<i>P_{lac}-T18-leucine zipper region from yeast GCN4. bla</i>	4	
<i>PUT18C-pbp2b</i>	<i>P_{lac}-T18-PBP2b bla pUC ori</i>	5	
<i>PUT18C-pbp2x</i>	<i>P_{lac}-T18-PBP2x bla pUC ori</i>	This study	43, 44
<i>PUT18C-PBP2x/PBP2bT M-CD</i>	<i>P_{lac}-T18-PBP2b(Met1-Tyr38)-PBP2x(Thr52-Asp750) bla pUC ori</i>	This study	45, 46, 48, 49
<i>PUT18C-PBP2b/PBP2xT M-CD</i>	<i>P_{lac}-T18-PBP2x(Met1-Gly51)-PBP2b(Met39-Asn685) bla pUC ori</i>	This study	47, 48, 49, 50
<i>PUT18C-pbp1a</i>	<i>P_{lac}-T18-PBP1a bla pUC ori</i>	6	
<i>PUT18C-pbp2a</i>	<i>P_{lac}-T18-PBP2a bla pUC ori</i>	6	
<i>PUT18C-PBP2a/PBP1aT M-CD</i>	<i>P_{lac}-T18-PBP1a(Met1-Ser37)-PBP2a(Ser79-Arg731) bla pUC ori</i>	This study	51, 52, 53, 54

<i>PUT18C-PBP1α/PBP2aT M-CD</i>	<i>P_{lac}-T18-PBP2a(Met1-Lys78)-PBP1α(Lys38-Pro719) bla pUC ori</i>	This study	48, 55, 56, 57
<i>pKT25-copD</i>	<i>P_{lac}-T25-copD kan pACYC ori</i>	This study	57, 58, 59, 60

References

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- 2 - Bullock, W. O., Fernandez, J. M. & short, J. M. A high efficiency plasmid transforming *recA Escherichia coli* strain with beta-galactosidase selection. *Biotechniques* 5, 376 (1987).
- 3 - Studier, F. W. & Moffatt, B. A. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J Mol Biol* 189, 113-130 (1986).
- 4 - Karimova G, Ullmann A, Ladant D. Protein-Protein Interaction Between *Bacillus stearotherophilus* Tyrosyl-tRNA Synthetase Subdomains Revealed by a Bacterial Two-Hybrid System. *J Mol Microbiol Biotechnol* 3(1):73–82 (2001).
- 5 - Straume, Stamsås, Berg, Salehian, Håvarstein. Identification of pneumococcal proteins that are functionally linked to penicillin-binding protein 2b (PBP2b). *Molecular Microbiology* (2017) 103(1), 99–116 (2016).
- 6 - Fenton AK, Mortaji LE, Lau DTC, Rudner DZ, Bernhardt TG. CozE is a member of the MreCD complex that directs cell elongation in *Streptococcus pneumoniae*. *Nature Microbiology* 2(0):1–9 (2016).

Table S2 - List of primers

Number	Name	Sequence 5'-3'
1	Upstream of <i>copD</i> (-635) (+)	CACTGTACCAGGGATAGAGAAG
2	Downstream of <i>copD</i> (+1134) (-)	CACCATTGATGACCACTGTTTGC
3	Janus cassette (+)	CCGTTTGATTTTTAATGGATAATG
4	Janus cassette (-)	AGAGACCTGGGCCCTTTCC
5	Janus / Upstream of <i>copD</i> (-1) (-)	CATTATCCATTA AAAATCAAACGGCCCT AGACCATACTGCCACAG
6	Janus / Downstream of <i>copD</i> (+488) (+)	GGAAAGGGGCCAGGTCTCTGGTCAAT AAGGAGGGCCTATG
7	Upstream of <i>copD</i> (-1) (-)	CCCTAGACCATACTGCCACAG
8	Downstream of <i>copD</i> (+411) (+)	CTGTGGCAGTATGGTCTAGGGGGTCAA TAAGGAGGGCCTATG
9	<i>copD-ΔPepSY</i> (+)	AACTATCCAACCGTGTT
10	<i>copD-ΔPepSY</i> (-)	ACACGGTTGGATAGTTTCAATACTGCTG AGCT
11	Linker / <i>copD</i> (+429) (-)	CTGCAGGAACCTCGATGTCTAGTTTTAGG CCCTCCTATTGACC

12	<i>sfGfp</i> (-)	TTATTTGTAGAGCTCATCCATGCCG
13	<i>copD</i> / <i>sfGfp</i> (+)	CGGCATGGATGAGCTCTACAAATAAGG TCAATAAGGAGGGCCTATG
14	<i>cpsN</i> (+308) (+)	CATCGGAACCTATACTCTTTTAG
15	<i>cpsO</i> (+800) (-)	ATAACAAATCCAGTAGCTTTGG
16	P_{comX} (0) (+)	TGAACCTCCAATAATAAATAAATAAAT
17	<i>cpsN</i> /0 (+1106) (+)	TTTCTAATATGTAACCTCTCCCAAT
18	<i>cpsN</i> / <i>copD</i> (-)	ATTGGGAAGAGTTACATATTAGAAATCA TAGGCCCTCCTTATTGAC
19	P_{comX} / <i>copD</i> (+)	ATTTATATTTATTATTGGAGGTTCAATGA AAAAAAGACAAAACAAAGCAAAAAATA ATC
20	P_{comX} / <i>sfGfp</i> (+)	TATTTATTATTGGAGGTTCAATGTCTAAA GGTGAAGAGTT
21	<i>cpsN</i> / <i>sfGfp</i> (-)	GAAGAGTTACATATTAGAAATTATTTGT ACAATTCATCCA
22	Upstream of <i>PBP2b</i> (-658) (+)	GCCGCTTGGTCTGTCAAAGCTTC
23	Downstream of <i>PBP2b</i> (+2725) (-)	CACTATACAAAAGAGGCCGATAGG
24	<i>gfp</i> / linker (-)	TCCGGATCCCTCGAGTTTATACAATTCAT CCATACCATGTG
25	Linker / <i>PBP2b</i> (+)	CTCGAGGGATCCGGAATGAGACTGATT TGTATGA
26	Upstream of <i>PBP2b</i> / <i>gfp</i> (+)	AGATTTTAAGTTAGAAATGATTTCTAAA GGTG
27	<i>rfp</i> / linker (-)	TCCGGATCCCTCGAGCAATTTACTAGGC AAAT
28	Upstream of <i>PBP2b</i> / <i>rfp</i> (+)	AGATTTTAAGTTAGAAATGAAACATCTT ACCG
29	P_{comX} / <i>ezrA</i> (+)	ATTTATATTTATTATTGGAGGTTCAATGT CTAATGGACAATAATTTATTTAATGG
30	<i>cpsN</i> / <i>ezrA</i> (-)	ATTGGGAAGAGTTACATATTAGAAATTA AAAACGAATCGTTTCACGTGTTTTTC
31	Upstream of <i>ezrA</i> (-587) (+)	CAGCCATGGGAACAGGATTTG
32	Downstream of <i>ezrA</i> (+2338) (-)	TTTATGATATTTAGCTGTACGAGAGTC
33	Upstream of Janus / <i>ezrA</i> (-)	CATTATCCATTAATAAATCAAACGGTTAA AAACGAATCGTTTCACGTGTTTTTC
34	Downstream of <i>ezrA</i> / Janus (+)	GGAAAGGGGCCAGGTCTCTTAAAAGA AAAAGATTTTATTGTGTGAGG
35	Downstream of <i>erzA</i> (+)	CGATTGTTTCAAGTAAAAAAGGAGTTTG ATTAAGAAAAAGATTTTATTGTGTGA GGAGC
36	Upstream of <i>ezrA</i> (-)	GCTCCTCACACAATAAAATCTTTTTCTTT TAATCAAACCTCTTTTTTACTTGAAACAA TCG
37	Extracellular domain of <i>copD</i> with BamHI extension (-)	TATGGATCCTCATAGGCCCTCCTTATTGA CC
38	Extracellular domain of <i>copD</i> with NdeI extension (+)	TATCATATGAAACCTATCAAACAGCTA AAAG
39	<i>PBP2b</i> with BamHI extension (-)	TATGGATCCCTAATTCATTGGATGGTAT TTTTGATACAG

40	<i>PBP2b</i> with NdeI extension (+)	GGGAATCCATATGCAGGTTTTGAACAA GGATTTTTACG
41	<i>PBP1a</i> with BamH1 extension (-)	TATGGATCCTTATGGTTGTGCTGGTTGA G
42	<i>PBP1a</i> with NdeI extension (+)	GGGAATCCATATGAGCAAGGCTCCTA GCCTATCCG
43	<i>PBP2x</i> with Acc65I extension (-)	TATGGTACCCGTTAGTCTCCTAAAGTTA ATGTAATTTTTTAATG
44	<i>PBP2x</i> with XbaI extension (+)	TATTCTAGAGATGAAGTGGACAAAAG AGTAATC
45	<i>PBP2b</i> (M1-Y38) fused to <i>PBP2x</i> (T52-D750) (+)	TTGGTCGTTTGTGTATACTCGCTTTGGA ACAGA
46	<i>PBP2b</i> (M1-Y38) fused to <i>PBP2x</i> (T52-D750) (-)	TCTGTTCCAAAGCGAGTATACAACAAAC GACCAA
47	pKNT25/pUT18/pUT18C-GIBSON-XbaI (-)	CTCTAGAGTCGACCTGCAG
48	pKNT25/pUT18 (+)	GTATGTTGTGTGGAATTGTGAGCGG
49	<i>PBP2x</i> (M1-M51) fused to <i>PBP2b</i> (M39-N685) (+)	TCATTATTGGGACAGGCATGCAGGTTTT GAACAA
50	<i>PBP2x</i> (M1-M51) fused to <i>PBP2b</i> (M39-N685) (-)	TTGTTCAAACCTGCATGCCTGTCCCAAT AATGA
51	<i>PBP1a</i> (M1-S37) fused to <i>PBP2a</i> (S79-R731) (+)	TTTTCTACTACGTTAGCTCGACCAATGTC AATGA
52	<i>PBP1a</i> (M1-S37) fused to <i>PBP2a</i> (S79-R731) (-)	TCATTGACATTGGTCGAGCTAACGTAGT AGAAAA
53	pUT18C (-)	TCGGGGCTGGCTTAACTA
54	pKNT25/pUT18C/pUT18 (+)	GTACCGAGCTCGAATTCA
55	<i>PBP2a</i> (M1-L78) fused to <i>PBP1a</i> (S38-P719) (+)	TGTTTGCTGTAGCCAAGAAGGCTCCTAG CCTATC
56	<i>PBP2a</i> (M1-L78) fused to <i>PBP1a</i> (L38-P719) (-)	GATAGGCTAGGAGCCTTCTTGGCTACAG CAAACA
57	pKT25-GIBSON-XbaI (-)	CTCTAGAGTCGACCCTGCAG
58	pKT25-GIBSON-Acc65I (+)	CGGGTACCTAAGTAACTAAGAATTC
59	<i>copD</i> with XbaI extension (-)	AGGGTCGACTCTAGAGGTGAAAAAAG ACAAA
60	<i>copD</i> with Acc65I extension (-)	TATGGTACCCGTAGGCCCTCCTTATTGA CCAATG